

The Effects of Clobazam and Diazepam on Central GABAergic System

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ABSTRACT

The interaction between clobazam or diazepam, and various GABAergic drugs (muscimol, baclofen, thiosemicarbazide) with regard to their effects on locomotor activity were studied. The locomotor activity produced by clobazam 1 mg/kg was not altered by GABA receptor agonist muscimol or baclofen, while muscimol or baclofen potentiated the depressive effects of clobazam (10 mg/kg), and diazepam (1 and 10 mg/kg). Furthermore, a reduction of clobazam (10 mg/kg), or diazepam (1 and 10 mg/kg)-induced suppression was seen upon administration of the GABAergic antagonist thiosemicarbazide. Clobazam is less effective than diazepam in increasing GABAergic activity. It is concluded that the reasons why clobazam cause less side effects than diazepam may be due to weaker effect on GABA-mediated inhibition in the central nervous system.

INTRODUCTION

Benzodiazepines are widely used clinically for their anxiolytic, anticonvulsant, sedative and muscle relaxant effect. They have not only central actions but also peripheral effects, although the evidence for the peripheral actions has not been fully established. The actions of benzodiazepines are on the central nervous system either by blocking the synthesis, turnover, release or metabolism of central monoamines neurotransmitters, or inhibiting the action of central monoamines neurotransmitters at postsynaptic receptors. Recently the claim of GABA in at least some central action of benzodiazepines is a new and interesting speculation.

In our previous study¹, using aminooxyacetic acid (AOAA), a compound which increase the level of GABA in the central nervous system by inhibiting GABA- α -ketoglutaric acid transaminase², and picrotoxin, a GABA receptor blocker³, we found that clobazam is less effective than diazepam in increasing GABAergic activity. However, picrotoxin has some convulsive effect which may interfere with the observation on locomotor activity⁴, and several investigators noted discrepancies between the time courses of AOAA's anticonvulsant effects and its brain GABA elevation effects⁵. Therefore, this study was carried out to investigate the relationship between clobazam-induced loco-

motor inhibition and GABAergic activity, and compared with diazepam by using muscimol⁶ and baclofen⁷, two GABAergic agonists, and thiosemicarbazide⁸, a GABAergic antagonist.

MATERIALS AND METHODS

1. Animals:

Male Sprague-Dawley rats, weighing between 190 to 220 g were used throughout these studies. The animals were maintained in groups of five per cage at a controlled temperature of $22 \pm 1^\circ\text{C}$, and housed in a metal-wire mesh cage for at least 7 days before each experiment. A solid diet Rodent Lab Chow (Ralston Purina Company U.S.A.) and tap water were provided ad libitum.

2. Drugs and treatment regimens:

The following compounds were used in these experiments: clobazam (CBZ, Hoechst Japan), diazepam (DZP, Hoffmann-La Roche), muscimol HBr (Sigma), baclofen (Daiichi Co., Japan) and thiosemicarbazide (Thio, Aldrich). All compounds were injected intraperitoneally in a volume of 2 ml/kg body weight, thiosemicarbazide and clobazam or diazepam were administered concomitantly to one rat, they were injected several second apart into different intraperitoneal sites, muscimol or baclofen were given before clobazam or diazepam every 10 min. Control rats received injection of the appropriate vehicle 0.5% CMC. All compounds were suspended in 0.5% CMC solution.

3. Locomotor activity measurements

The locomotor activity of individual rats was measured using the MK-Animex (Muromachi KiKai Co., Japan). The sensitivity and turning of the instrument was adjusted to 35 uA to enable all kinds of motor behavior, including locomotion, rearing, grooming, and sniffing to be jointly recorded. Activity was recorded for 60 min. intervals for 3 hr, starting 5 min after inserting the animals into the test cage. Each rat was used only once.

4. Statistical analysis

Results were analyzed statistically using Student's-t-test to compare intergroup differences.

RESULTS

1. Effect of muscimol on the locomotor inhibition produced by clobazam and diazepam.

As shown in Figure 1, pretreatment of rats with GABA receptor agonist muscimol in a dose of 0.5 mg/kg showed a slight but significant decrease in the motility ($p < 0.05$), compared to the motility of untreated control animals. The locomotor activity produced by clobazam (1 mg/kg) was not altered by muscimol, but clobazam (10 mg/kg)-induced inhibition was enhanced by muscimol during the first 60-min. session ($p < 0.05$). However, hypomotility produced by diazepam (1 and 10 mg/kg) was markedly enhanced between 60 and 180 min. by muscimol ($p < 0.05$ to $p < 0.01$).

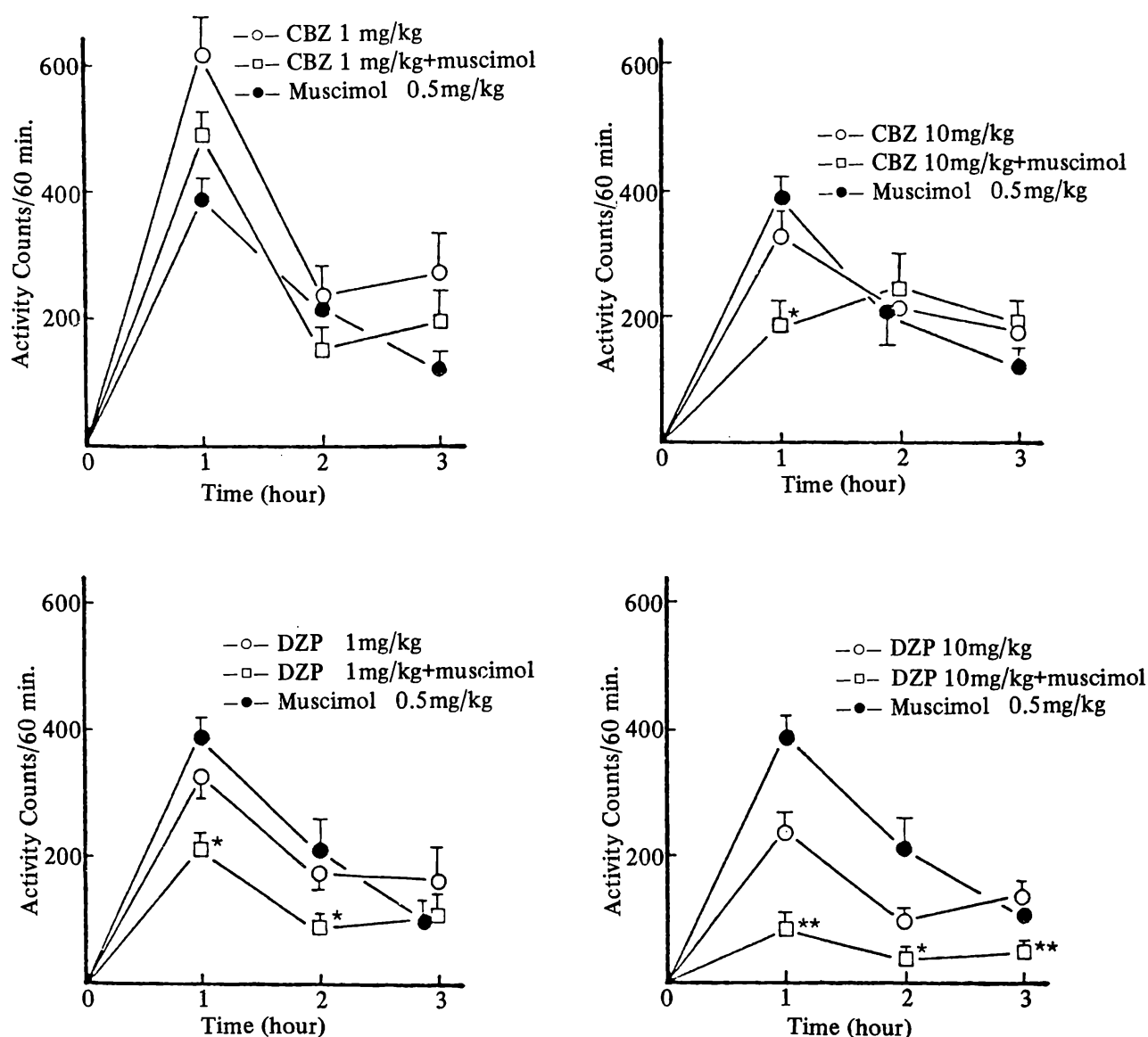


Fig. 1. Time course of the effect of muscimol 0.5 mg/kg alone, and muscimol 0.5 mg/kg in combination with clobazam, or diazepam on locomotor activity in rats. The values shown are the means and S.E. (vertical bars). Each point represents the mean of 6 independent observations. The asterisks indicate values that are significantly different from the corresponding results for clobazam, or diazepam administration, respectively. (* $p < 0.05$, ** $p < 0.01$).

2. Effect of baclofen on the locomotor inhibition produced by clobazam and diazepam.

As shown in Figure 2, rats pretreated with 0.5 mg/kg of GABA receptor agonist baclofen showed a slight but significant decrease in the motility ($p < 0.05$), compared to the motility of untreated control animals. The locomotor activity produced by clobazam (1 mg/kg) was not altered by baclofen, but hypomotility produced by clobazam (10 mg/kg) was markedly enhanced by baclofen during the first 60-min session ($p < 0.01$). On the other hand, hypomotility produced by diazepam (1 and 10 mg/kg) was markedly enhanced between 60 and 180 min. by baclofen administration ($p < 0.05$ to $p < 0.01$).

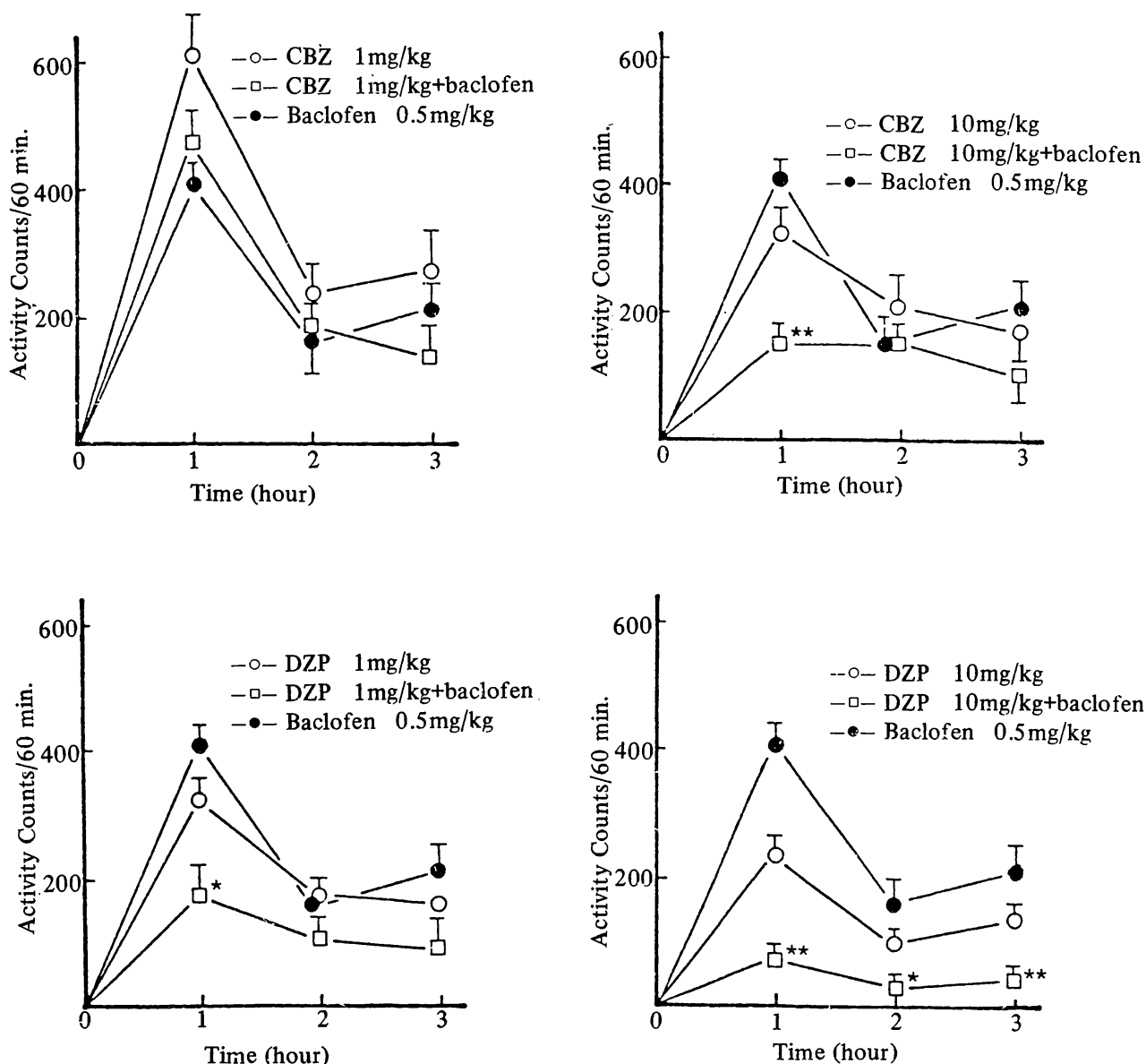


Fig. 2. Time course of the effect of baclofen 0.5 mg/kg alone, and baclofen 0.5 mg/kg in combination with clobazam, or diazepam on locomotor activity in rats. For details see legend for Fig. 1. The asterisks indicate values that are significantly different from the corresponding results for clobazam, or diazepam administration, respectively. (* $p < 0.05$, ** $p < 0.01$).

3. Effect of thiosemicarbazide on the locomotor inhibition produced by clobazam and diazepam.

Rats treated with the GABAergic antagonist thiosemicarbazide (0.5 mg/kg) alone exhibited slightly decreased locomotion, but the degree was not statistically different from the 0.5% CMC control. As Figure 3 shows, clobazam (10 mg/kg)-induced suppression was significantly reversed between 60 and 120 min. by thiosemicarbazide administration ($p < 0.05$), but thiosemicarbazide did not alter the locomotor effect of clobazam (1 mg/kg), while diazepam (1 and 10 mg/kg)-induced suppression was markedly and significantly reverse between 60 and 120 min by thiosemicarbazide administration ($p < 0.05$ to $p < 0.001$).

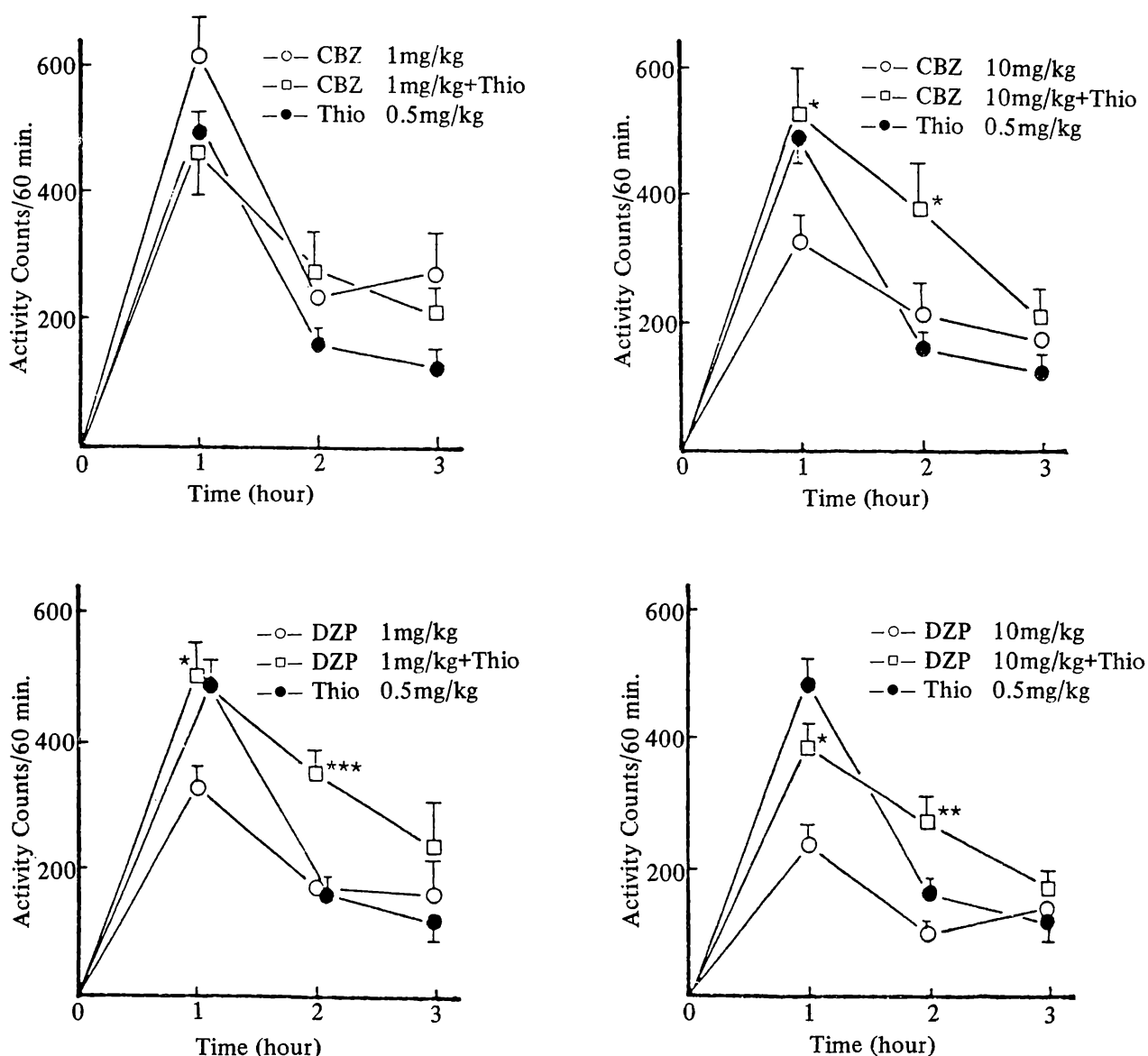


Fig. 3. Time course of effect of thiosemicarbazide 0.5 mg/kg alone, and thiosemicarbazide 0.5 mg/kg in combination with clobazam, or diazepam on locomotor activity in rats. For details see legend for Fig. 1. The asterisks indicate values that are significantly different from the corresponding results for clobazam, or diazepam administration, respectively. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

DISCUSSION

The ever-increasing tension in our everyday life accompanying the industro-economic development increases the incidence of anxiety neurosis. Benzodiazepines are widely used clinically, because they are shown to be efficacious and well tolerated anxiolytic agents in various neurotic and psychosomatic disorders. However, because of the abuse of these agents, more and more side effects were discovered. Among these side effects are ataxia, hostility, and irritability. A large number of anxiolytic agents of similar chemical structure have been developed to try to increase the anxiolytic effect and decrease the side effects.

Clobazam is a new anxiolytic agents, which belongs to the class of the 1,5-benzodiazepines.

Although its mode of action has been studied by many researchers, the mechanism in the central nervous system has not yet been clearly defined. According to Rastogi⁹, monoamines in the central nervous system played an important role in behavioral excitation. And decreasing the monoamines levels in the CNS might be responsible for the tranquilizing effects of benzodiazepines. His study only mentioned the relationship between benzodiazepine and monoamines, but not involved the discussion between benzodiazepines and GABA.

In our previous study^{1,10}, the relationship between benzodiazepines and both monoamines and GABA was discussed. In studying the relationship between benzodiazepines and GABA, we used AOAA, an inhibitor of GABA-transaminase, and picrotoxin, an GABA receptor antagonist. However, picrotoxin was reported to cause convulsion, which may interfere with the observation on locomotor activity⁴. And several investigators noted discrepancies between the time course of AOAA's anticonvulsant effects and its brain GABA elevation effects⁵.

In the present study we used another two GABA agonist, muscimol and baclofen, and one GABA antagonist, thiosemicarbazide to study the relationship between clobazam and GABA, and compared with diazepam.

In studying the relationship between benzodiazepines and GABA, there are quite a few methods, e.g., biochemical method¹¹, iontophoresis¹², tissue culture¹³, and locomotor activity method¹⁴. Locomotor activity meter involve least expenses and no special technique is required. The assessment of motor activity is quite accurate and sensitive. Up to date, there are quite a few reports, using locomotor activity meter, to study the relationship between GABA and drugs¹⁵. However, the relationship between clobazam and GABA, using the locomotor activity, has not been studied. In this report, we provide an easy technique, using the locomotor activity meter to study the relationship between clobazam and GABA.

In our previous study^{1,10}, we used Animex Activity Meter Type-S (Sweden) to study the motor activity changes after giving clobazam and diazepam respectively. In the present study, we used MK-Animex (Japan) to study the motor activity. The results agreed with those of Fielding¹⁶, diazepam was more active than clobazam in reducing locomotor activity and clobazam could only reduce motor activity at higher doses.

The muscimol used in the present experiment is considered as a GABA agonist which stimulates the GABA receptor⁶, when muscimol was given alone, it appeared to have slight behavioral depressive effects. When muscimol was given along with clobazam (10 mg/kg), the behavioral depressive effect was potentiated at 60 min. However, the behavioral depressive effect produced by diazepam (1 and 10 mg/kg) was markedly enhanced between 60 min and 180 min by muscimol. The baclofen was considered to be a GABA agonist⁷, although its specificity had ever been questioned. When baclofen was used alone, it appeared to have slight behavioral depressive effect, when baclofen was given along with clobazam or diazepam, the depressive effect was potentiated. These results suggest the viewpoint that benzodiazepines increase the GABA mediated inhibition in the CNS to exhibit their sedative properties¹³.

The thiosemicarbazide was considered to be a GABA antagonist, benzodiazepines given after AOAA further enhanced presynaptic inhibition and increase the dorsal root potential. However, when benzodiazepines was given after thiosemicarbazide, its usual effect was completely absent¹⁸. The present experiment shows, clobazam (10 mg/kg), or diazepam (1 and 10 mg/kg)-induced sup-

pression was markedly and significantly reversed by thiosemicarbazide. The results agree with those of Polc¹⁸, thiosemicarbazide can antagonize the benzodiazepine effect.

Benzodiazepines can specifically increase the GABA mediated inhibition in the CNS¹³, and selectively potentiate the effects of both exogenous and synaptically released GABA¹⁹. GABA is the transmitter that mediates the inhibition of Purkinje cell activity²⁰, also ataxia, muscle relaxation, behavioral depression, sedation caused by benzodiazepines are related to GABA²¹. Fielding pointed out that clobazam has a specific advantage over diazepam and chlordiazepoxide in that ataxia, muscle relaxation, sedation, and behavioral depression are at doses much higher than those necessary to produce anxiolytic and taming activity, and furthermore that clobazam possess effective anti-anxiety activity between diazepam and chlordiazepoxide without overlapping side effect¹⁶. He did not provide any explanation to the above phenomenon. The present experiment may suggest that the reasons why clobazam cause less side effects than diazepam may be due to weaker effect on GABA-mediated inhibition in the central nervous system.

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中 文 摘 要

Clobazam 及 Diazepam 對於中樞 GABA 系統之影響

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本研究為探討 clobazam 或 diazepam 與各種 GABA 作用藥物 (muscimol, baclofen, thiosemicarbazide) 間之相互作用，以測定大白鼠之自發運動量為指標。clobazam 1 mg/kg 所產生之運動量不受 GABA 致效劑 muscimol 或 baclofen 之影響，但 muscimol 或 baclofen 能增加 clobazam 10 mg/kg 及 diazepam (1, 10 mg/kg) 之運動量抑制作用。又 clobazam 10 mg/kg 或 diazepam (1, 10 mg/kg) 所產生之運動量抑制作用被 GABA 拮抗劑 thiosemicarbazide 所拮抗。clobazam 增強 GABA 之活性比 diazepam 弱。由本實驗結果顯示 clobazam 之臨床使用副作用少於 diazepam，可能與 clobazam 影響中樞 GABA 系統之抑制作用比 diazepam 弱有關。